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Short Communication

HMGI-C Expression Patterns in Human Tissues

Implications for the Genesis of Frequent Mesenchymal Tumors

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Cytogenetically visible aberrations of chromosomal region 12q14–15 in a variety of frequent benign human tumors reflect rearrangements of the HMGI-C gene. The mechanisms by which the HMGI-C gene contributes to tumorigenesis are mostly unknown, although frequently aberrant transcripts containing exons 1 to 3 of HMGI-C and ectopic sequences from other genes due to breaks within the third intron of HMGI-C are detectable. This is the first report analyzing human tissue samples mainly of mesenchymal origin by a highly sensitive polymerase-chain-reaction-based approach detecting HMGI-C expression. We found HMGI-C expression in embryonic tissue but no expression in any of several adult tissues tested except for two myometrial tissues. These data suggest that HMGI-C is mainly expressed in human tissues during embryonal and fetal development. Thus, its particular role for tumor development may be due to the expression of at least exons 1 to 3 rather than to the formation of fusion transcripts. (Am J Pathol 1996, 149:775–779)

Rearrangements of the HMGI-C gene assigned to 12q15[1] have recently been identified to underlie cytogenetically visible aberrations of chromosomal region 12q14–15 in a variety of benign human solid tumors, eg, pleomorphic adenomas of the salivary glands, uterine leiomyomas,[2–4] lipomas,[2–5] pulmonary chondroid hamartomas,[4,6,7] endometrial polyps,[8] aggressive angiomyxomas,[9] and fibroadenomas and adenolipomas of the breast.[10,11] Frequent alterations of that gene are fusion genes due to breaks within the third intron of the HMGI-C resulting in aberrant transcripts containing exons 1 to 3 of HMGI-C and ectopic sequences from other genes, eg, the 13th exon of the mitochondrial aldehyde dehydrogenase.[12] As most of the tumors affected are undoubtedly of mesenchymal origin, these findings suggest that the normal expression of the gene is linked to the development or differentiation of mesenchymal tissues.

This assumption is also supported by a recent observation by Zhou et al,[13] who were able to show that in mice the functional knock out of the HMGI-C gene results in the well known so-called pygmy phenotype with a characteristic hypoplasia of mesenchymal tissues, eg, smooth muscle and adipose tissue. In mice, the expression of HMGI-C has been studied in various fetal and adult tissues.[13] During mouse development, HMGI-C was expressed between as early as 10.5 days and 15.5 days post-coitum. Expression was observed in most tissues and organs during embryogenesis except for the brain where there was only expression in a small, localized region of the forebrain. However, the sensitivity of the latter study was limited by the Northern blot analyses and the *in situ* hybridization applied, and for human tissues, no comparable data are available

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Table 1. *Summary of the Results of the RT-PCR Assay for the Expression of HMGI-C in Several Human Tissues of Fetal and Adult origin*

Sample	Anatomical site of tissue	Diagnosis	Age	Expression of HMGI-C
Fetal tissue	Whole fetus	Induced abortion	8 weeks	+
Fetal tissue	Whole fetus	Induced abortion	9 weeks	+
Fetal tissue	Corpus and extremities	Induced abortion	10 weeks	+
Fetal tissue	Brain	Induced abortion	10 weeks	−
Fetal tissue	Corpus	Induced abortion	12 weeks	+
Fetal tissue	Head	Induced abortion	12 weeks	+
Newborn				
Placenta	Fetal placenta	Delivery at term	36 weeks	−
Placenta	Maternal placenta	Delivery at term		−
Umbilical cord		Delivery at term	36 weeks	−
Adult				
Adipose tissue	Groin	Suspect lymph node	73 years	−
Adipose tissue	Vulva, subcutaneous	Invasive carcinoma	73 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	54 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	46 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	48 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	47 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	48 years	+
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	44 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	54 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	42 years	+
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	58 years	−
Smooth muscle	Uterus	Hysterectomy (uterus myomatosus)	60 years	−
Muscle	Heart, right ventricle	Heart transplantation	53 years	−
Breast tissue	Breast	Benign breast disease	57 years	−
Breast tissue	Breast	Invasive ductal breast cancer	57 years	−
Breast tissue	Breast	Benign breast disease	45 years	−
Breast tissue	Breast	Benign breast disease	52 years	−
Breast tissue	Breast	Benign breast disease	31 years	−
Breast tissue	Breast	Invasive ductal breast cancer	55 years	−
Vein	Vena cava	Heart transplantation	53 years	−
Tube; ovarian cyst	Tube and ovary	Ovarian cyst	51 years	−
Skin	Vulva	Invasive carcinoma	73 years	−
Lymph node	Groin	Invasive carcinoma	73 years	−
Salivary gland	Parotis gland	Pleomorphic adenoma	45 years	−
Salivary gland	Parotis gland	Pleomorphic adenoma	55 years	−
Lung tissue	Lung	Lung carcinoma	47 years	−
Lung tissue	Lung	Lung carcinoma	52 years	−

Most of the tissue samples from adult individuals are from adjacent normal tissue obtained during surgery of benign or malignant tumors.

yet. We have thus established a highly sensitive reverse transcriptase polymerase chain reaction (RT-PCR) assay to study the expression pattern of HMGI-C in several human tissues of fetal and adult origin. We have particularly addressed those tissues and organs from which tumors with HMGI-C mutations arise. The results allow for better insights as to whether alterations of the HMGI-C gene contribute to the development of very frequent human tumors.

Materials and Methods

Tissue Samples

All 27 adult tissue samples used for this study were taken from surgically removed tissue frozen in liquid nitrogen within a period of 15 minutes after removal. Details regarding the anatomical site of the tissue, the reason for removal, and age of patients are given in Table 1. Most of the samples were from adjacent normal tissue removed during tumor surgery. The 6 fetal tissues taken after induced abortions and the 3 newborn tissues taken from a delivery at term were prepared as described for the adult tissues.

In addition, three cell lines were used. As a control for HMGI-C expression, we used the hepatoma cell line Hep 3B and the cell line L14 established from a lipoma with the typical translocation t(3;12).[14] HeLa cells were used as a negative control because HMGI-C protein is essentially absent from HeLa[15] and RT experiments reproduced 10 times did not reveal HMGI-C expression in our own studies.

RT-PCR

A total of 100 mg of tissue sample was homogenized, and RNA was isolated using the trizol reagent (Gibco BRL, Eggenstein, Germany) containing phenol and isothiocyanate. cDNA was synthesized using a poly(A)-oligo(dt)17 primer and M-MLV reverse transcriptase (Gibco BRL). A hemi-nested PCR was then performed. For the first- and second-round PCR, the same lower primer (Revex 4) 5′-TCC TCC TGA GCA GGC TTC-3′ (exon 4/5) was used. In the first round of PCR, the specific upper primer (SE1) 5′-CTT CAG CCC AGG GAC AAC-3′ (exon 1) was used, and in the second round of PCR, the nested upper primer (P1) 5′-CGC CTC AGA AGA GAG GAC-3′ (exon 1) was used.

Both rounds of PCR were performed in a 100-μl volume containing 10 mmol/L Tris/HCl pH 8.0, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.001% gelatin, 100 μmol/L dATP, 100 μmol/L dTTP, 100 μmol/L dGTP, 100 μmol/L dCTP, 200 nmol/L upper primer, 200 nmol/L lower primer, and 1 U/100 μl AmpliTaq polymerase (Perkin Elmer, Weiterstadt, Germany). Amplification was performed for 30 cycles (1 minute at 94°C, 1 minute at 53°C, 2 minutes at 72°C). As template in the first round of PCR, cDNA derived from 250 ng of total RNA was used, and in the second round of PCR, 1 μl of the first PCR reaction mix was used.

The PCR products were separated on 1.2% agarose gels, blotted, and hybridized with a 220-bp HMGI-C cDNA probe. This probe was generated and labeled by a PCR reaction using the same primers as described above for the second round of RT-PCR. A HMGI-C cDNA obtained by 3′RACE-PCR product[12] served as template. Hybridization and detection were performed as described by Rogalla et al[16] with the following modifications: hybridization temperature was 40°C overnight followed by the stringent wash at 65°C.

Control and Sensitivity Assays

As control reaction for intact RNA and cDNA, a PCR according to Baier et al[17] with some minor modifications was performed. This test is based on the amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR reaction was performed for 35 cycles under the same conditions as described above for the first round of PCR of HMGI-C expression.

For determination of the sensitivity of the PCR-based detection of HMGI-C gene expression, cell lysates from Hep 3B cells were mixed with those from HeLa cells. Cells were lysed in a culture flask containig approximately 10^6 cells by using 600 μl of the trizol reagent (Gibco BRL), and cell lysates were directly used for mixtures. The following procedures were performed as described above.

Results

As for the expression studies, all experiments were repeated at least twice. As the result of RT-PCR in HMGI-C-positive cells such as Hep 3B and L14, a specific 220-bp fragment was detectable (Figure 1a), the origin of which was clearly confirmed after hybridization (Figure 1b). HeLa cells did not show an expression of HMGI-C. After RT-PCR, a few nonspecific fragments were detectable (Figure 1a) that did not hybridize to the HMGI-C probe (Figure 1b).

To determine the sensitivity of the assay, Hep 3B cells were mixed with HeLa cells at the following ratios: 1:4, 1:10, 1:20, 1:200, 1:2,000, 1:20,000, and 1:200,000. The results revealed that HMGI-C expression was detectable up to 1 positive of 2,000 cells but is not detectable at a ratio of 1:20,000 (Figure 1, a and b).

The data of HMGI-C expression of different cells are summarized in Table 1. Except for two myometrial samples (Figure 2), all normal tissue samples taken from adult individuals did not show any detectable level of HMGI-C expression. Also, no expression was noted in placental tissue or in umbilical cord from a delivery at term.

In contrast, except for the brain, the embryonal tissues tested revealed HMGI-C expression.

To assure that all RNA and cDNA preparations used for the RT-PCRs were intact (otherwise resulting in false negative results), a RT-PCR for expression of the housekeeping gene GAPDH was performed routinely. A positive GAPDH RT-PCR resulted in a 299-bp fragment (Figure 1c). Only those samples revealing a positive GAPDH RT-PCR were used for this study.

Discussion

There is currently a lot of evidence that rearrangements of the HMGI-C gene are causally related to the development of frequent benign tumors. Often, intragenic rearrangements of one allele leading to fusion genes have been observed,[4,5,7,12] but in all cases studied so far the second apparently normal allele was found to be expressed as well. This suggests that HMGI-C is also expressed in the normal tissue from which the tumor originates. However, studies on HMGI-C expression in mice based on Northern blot

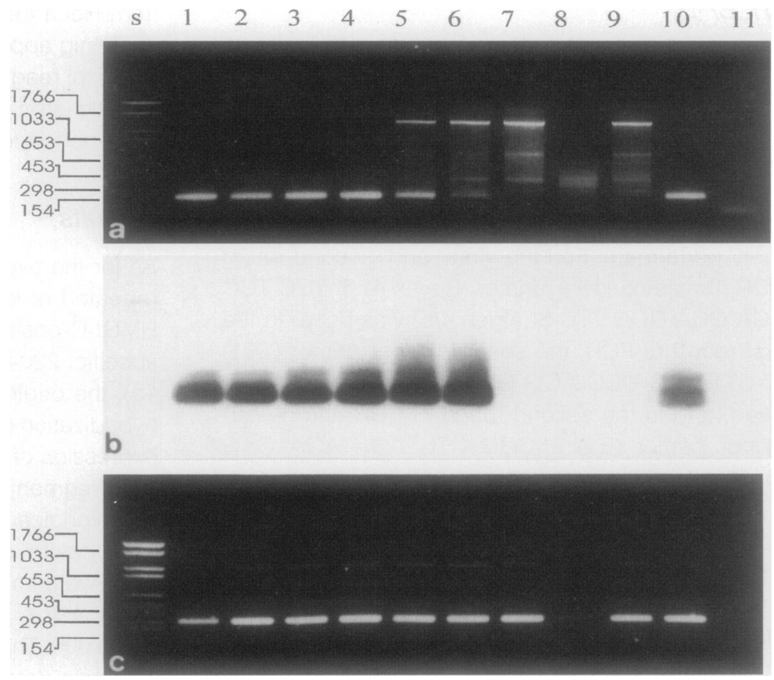

Figure 1. *Determination of sensitivity of the PCR-based detection of HMGI-C gene expression. Hep 3B cells were mixed with HeLa cells at the following ratios: Hep 3B cells only* (lane 1), *1:4* (lane 2), *1:10* (lane 3), *1:20* (lane 4), *1:200* (lane 5), *1:2,000* (lane 6), *1:20,000* (lane 7), *1:200,000* (lane 8), *HeLa cells only* (lane 9). Lanes 10 and 11, *positive (L14) and negative (aqua bidest) control, respectively;* lane s, *DNA molecular weight standard VI (Boehringer Mannheim).* a: *RT-PCR products of HMGI-C after gel electrophoresis and ethidium bromide staining.* b: *Detection of specific HMGI-C fragments after Southern blot hybridization.* c: *RT-PCR products of GAPDH after gel electrophoresis and ethidium bromide staining.* Lane 8 *reveals a low concentration of amplification product.*

and *in situ* hybridization failed to detect any expression after the fetal period.[13] Because the situation could be different in human tissues and because of the lower sensitivity of the latter method, we have developed a more sensitive RT-PCR assay and applied it to several human tissue samples.

The results coincide with those obtained for the mice. There was an expression in fetal tissue except for the brain and placenta. With the exception of two myometrial samples, HMGI-C expression was noted in neither of the adult tissues. Both latter myometrial samples were from a uterus myomatosus and can thus most likely be explained by the presence of small myomas in an early phase of development or by contamination by larger myomas because all other myometrial samples were negative. Thus, the highly sensitive approach applied herein reveals that HMGI-C is in fact a protein more or less exclusively expressed in the fetal period. It is therefore tempting to speculate that, besides the formation of fusion genes, even a simple transcriptional deregulation of the gene can lead to the development of tumors in a variety of mesenchymal tissues of the adult. Chromosomal breakpoints that have occasionally been mapped 3′ or 5′ of the HMGI-C gene further confirm this assumption. It can accordingly be speculated that other genes fused to HMGI-C by intragenic rearrangements mainly act by providing enhancers rather than by contributing essential coding sequences. Following this idea, the expression of the second allele would be directly due to the expression of the former one as the cell is shifted back to a fetal stage, also resulting in the secondary activation of the normal and not the altered HMGI-C allele.

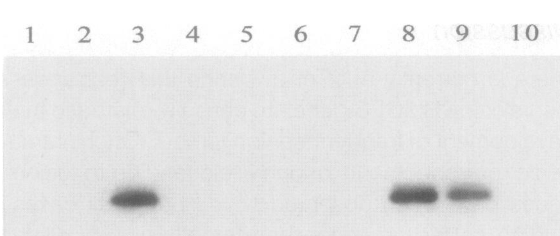

Figure 2. *HMGI-C gene expression of different tissue samples revealed by RT-PCR and Southern blot hybridization.* Lane 1, *negative control (HeLa);* lanes 2 *to* 6, *different myometrial samples;* lane 7, *endometrium;* lanes 8 *and* 9, *positive controls (Hep 3B and L14, respectively);* lane 10, *negative control (aqua bidest).*

In summary, the results of our study show that HMGI-C is mainly a protein of the embryogenic life. The data suggest that any type of HMGI-C mutation occurring in mesenchymal tissues leading to its activation and leaving intact at least exons 1 to 3 of the gene can induce the development of benign tumors, eg, leiomyomas, lipomas, or pulmonary chondroid hamartomas.

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References

1. Wanschura S, Kazmierczak B, Schoenmakers EFPM, Meyen E, Bartnitzke S, Van de Ven W, Bullerdiek J, Schloot W: Regional fine mapping of the multiple aberration region involved in uterine leiomyoma, lipoma, and pleomorphic adenoma of the salivary gland to 12q15. Genes Chromosomes & Cancer 1995, 14: 68–70
2. Van de Ven WJM, Schoenmakers EFPM, Wanschura S, Kazmierczak B, Kools P, Geurts JWM, Bartnitzke S, Van den Berghe H, Bullerdiek J: Molecular characterization of MAR, a multiple aberration region on human chromosome 12q13–15 implicated in various solid tumors. Genes Chromosomes & Cancer 1994, 12:296–303
3. Schoenmakers EFPM, Mols R, Wanschura S, Kools P, Geurts J, Bartnitzke S, Van den Berghe H, Bullerdiek J, Van de Ven W: Identification, molecular cloning, and characterization of the chromosome breakpoint cluster region of uterine leiomyomas. Genes Chromosomes & Cancer 1994, 11:106–118
4. Schoenmakers EFPM, Wanschura S, Mols R, Bullerdiek J, Van den Berghe H, Van de Ven W: Recurrent rearrangements in the high mobility group protein gene, HMGI-C, in benign mesenchymal tumours. Nature Genet 1995, 10:436–444
5. Ashar HR, Schoenberg Fejzo M, Tkachenko A, Zhou X, Fletcher JA, Weremowicz S, Morton CC, Chada K: Disruption of the architectural factor HMGI-C: DNA-binding AT hook motifs fused in lipomas to distinct transcriptional regulatory domains. Cell 1995, 82:57–65
6. Kazmierczak B, Wanschura S, Rosigkeit J, Meyer-Bolte K, Uschinsky K, Haupt R, Schoenmakers EFPM, Bartnitzke S, Van de Ven W, Bullerdiek J: Molecular characterization of 12q14–15 rearrangements in three pulmonary chondroid hamartomas. Cancer Res 1995, 55: 2497–2499
7. Kazmierczak B, Rosigkeit J, Wanschura S, Meyer-Bolte K, Van de Ven WJM, Kayser K, Krieghoff B, Kastendiek H, Bartnitzke S, Bullerdiek J: HMGI-C rearrangements as the molecular basis for the majority of pulmonary chondroid hamartomas: a survey of 30 tumors. Oncogene 1996 72:515–521
8. Bol S, Wanschura S, Thode B, Deichert U, Van de Ven WJM, Bartnitzke S, Bullerdiek J: An endometrial polyp with a rearrangement of HMGI-C underlying a complex cytogenetic rearrangement involving chromosome 2 and 12. Cancer Genet Cytogenet 1996 (in press)
9. Kazmierczak B, Wanschura S, Meyer-Bolte K, Caselitz J, Meister P, Bartnitzke S, Van de Ven W, Bullerdiek J: Cytogenetic and molecular analysis of an aggressive angiomyxoma. Am J Pathol 1995, 147:580–585
10. Rohen C, Caselitz J, Stern C, Wanschura S, Schoenmakers EFPM, Van de Ven WJM, Bartnitzke S, Bullerdiek J: A hamartoma of the breast with an aberration of 12q mapped to the MAR region by fluorescence *in situ* hybridization. Cancer Genet Cytogenet 1995, 84: 82–84
11. Staats B, Bonk U, Wanschura S, Hanisch P, Schoenmakers EFPM, Van de Ven WJM, Bartnitzke S, Bullerdiek J: A fibroadenoma with a t(4;12)(q27;q15) affecting the HMGI-C gene, a member of the high mobility group protein gene family. Breast Cancer Res Treat 1996, 38:299–303
12. Kazmierczak B, Hennig Y, Wanschura S, Rogalla P, Bartnitzke S, Van de Ven W, Bullerdiek J: Description of a novel fusion transcript between HMGI-C, a gene encoding for a member of the high mobility group proteins, and the mitochondrial aldehyde dehydrogenase gene. Cancer Res 1995, 55:6038–6039
13. Zhou X, Benson KF, Ashar HR, Chada K: Mutation responsible for mouse pygmy phenotype in the developmentally regulated factor HMGI-C. Nature 1995, 376:771–774
14. Belge G, Kazmierczak B, Meyer-Bolte K, Bartnitzke S, Bullerdiek J: Expression of SV40 T-antigen in lipoma cells with a chromosomal translocation t(3;12) is not sufficient for direct immortalization. Cell Biol Int Rep 1992, 16:339–347
15. Chau K-Y, Patel UA, Lee K-KD, Lam H-YP, Crayne-Robinson C: The gene for human architectural transcription factor HMGI-C consists of five exons each coding for a distinct functional element. Nucleic Acids Res 1995, 23:4262–4266
16. Rogalla P, Rohen C, Hennig Y, Deichert U, Bonk U, Bullerdiek J: Telomere repeat fragment sizes do not limit the growth potential of uterine leiomyomas. Biochem Biophys Res Commun 1995, 211:175–182
17. Baier G, Telford D, Gulbins E, Yamada N, Kawakami T, Altman A: Improved specificity of RT-PCR amplifications using nested cDNA primers. Nucleic Acids Res 1993, 21:1329–1330